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Stable production of *Aspergillus niger* β-galactosidase by delta-mediated chromosomal integration in flocculent *Saccharomyces cerevisiae*.

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This work aims to construct a stable producer flocculent *Saccharomyces cerevisiae* strain with *Aspergillus niger* β-galactosidase gene *lac*A using the repeated chromosomal delta yeast sequences as target sites for integration. Two different integration systems were used: the first one uses as dominant selection marker the G418 antibiotic resistance and allows for tandem integrations, while the second one uses the *URA*3 selection marker and integration occurs at different sites with one single copy of the gene at each site. Both integration systems resulted in recombinant *S. cerevisiae* strains that produce extracellular β-galactosidase. The most promising transformants were characterized genetically by Southern analysis. For the first system in maximum 8 *lac*A tandem copies were integrated, using G418 concentrations raging from 0.2 to 1.5 g/l, while for the second system, after 3 rounds of transformation and *URA*3 gene loss, only 2 dispersed copies were observed in the genome. Transformants belonging to the first system presented β-galactosidase activity in YPD medium comparable to the reference episomal based plasmid strain (Domingues *et al*., Appl Microbiol Biotechnol 58:645-650, 2002) and revealed integration stability, checked by Southern analysis, even after 80 generations. The stability of these transformants will allow for its use in a continuous high cell density system leading to increased β-galactosidase productivity.

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